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14. ABSTRACT
Funding of this multidisciplinary training program supports efforts to 1) use Random Periareolar Fine Needle Aspiration (RPFNA) to test for correlations between a) hyper-methylation of estrogen responsive genes b) estrogen responsive protein expression by immunohisto chemistry (IHC) and a cytological response to tamoxifen chemoprevention and then 2) test whether resistance to tamoxifen correlates with lack of co-activator recruitment to an estrogen response element (ERE) in state-of-the-art preclinical models.
We have established and validated methylation specific PCR primers for the detection of two isoforms of the Progesterone Receptor in Random Periareolar Fine Needle Aspiration (RPFNA) samples. Likewise we have developed protocols to evaluate the steroid hormone concentration in RPFNA samples. Preliminary investigation of steroid concentration indicates that there may be a positive correlation between breast tissue estradiol concentration and cytological atypia.

15. SUBJECT TERMS
RPFNA, Estradiol, Progesteron Receptor, Estrogen Receptor, GC-MS

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INTRODUCTION:

Funding of this multidisciplinary training program supports efforts to 1) use Random Periareolar Fine Needle Aspiration (RPFNA) to test for correlations between a) hypermethylation of estrogen responsive genes b) estrogen responsive protein expression by immunohisto chemistry (IHC) and a cytological response to tamoxifen chemoprevention and then 2) test whether resistance to tamoxifen correlates with lack of co-activator recruitment to an estrogen response element (ERE) in state-of-the-art preclinical models. This area of investigation is highly relevant to improving breast cancer prevention. By defining the molecular markers that predict response to tamoxifen chemoprevention, we can rapidly identify women who will or will not have a clinical benefit from tamoxifen. **Major findings to date and progress in fulfillment of Specific Aim I: Year 1 findings:** We have been investigating the role of methylation of estrogen responsive genes to predict cytological changes to tamoxifen chemoprevention. Research into the role of estrogen receptor alpha methylation indicated that there was likely a unique and important reciprocal relationship between estrogen receptor (ER) methylation and progesterone receptor (PR) expression. Therefore, in addition to validation of the estrogen receptor methylation studies we also designed and tested methylation specific primers to study the effects of PR hypermethylation on tamoxifen sensitivity. **Specific Aim 2: Year 1 findings.** In order to improve our ability to examine changes in estrogen response for cells isolated from RPFNA samples it was necessary to understand the hormonal conditions present in the breast microenvironment surrounding these cells. Therefore, we have developed a standard protocol for the evaluation of estradiol and triglyceride from RPFNA samples. Additionally, we recognized that the breast tissue contains a complex hormonal environment which provides a significant potential for hormone receptor cross-talk so we have begun development of a mass-spectrometry based system to analyze multiple steroids simultaneously from our RPFNA samples. The steroid profile information is critical to conducting biologically appropriate studies of the changes in recruitment of proteins to specific response elements. **Study Protocol: Human Subject Protocol Approval**. In March of 2007 we received final approval to use our human subject derived samples. At that time we began our evaluation of RPFNA derived samples and will have data to report in our Year 2 Findings in 2008.

BODY:

SPECIFIC AIM I: ***Does ER expression and/or hypermethylation of the ER α promoter* (ESR1) *predict cytological response to Tam chemoprevention?***

Progesterone Receptor promoter methylation profiling: Progesterone receptors (PR) are members of the nuclear receptor superfamily and are required for normal mammary gland development. The two PR isoforms, PR-A and PR-B, are produced from a single gene by translation initiation at two distinct start codons under the control of separate promoters. Normal breast express equimolar ratios of PR-A: PR-B, however, this is altered by hormone replacement therapies, E and E+P, and use of tamoxifen in cancer treatments. PR-A excess has been associated with poor clinical outcome and with more rapid disease recurrence after tamoxifen treatment. The status of ER and PR can change over the natural history of the disease or during treatment. PR levels decrease more dramatically during tamoxifen therapy, with up to half of tumors completely losing PR expression when resistance develops. Emerging evidence suggests that the relation between gene methylation and protein expression for ER and PR's is more complicated than that of other genes. The current hypothesis is that methylation of PR gene may be a better predictor of ER expression and that methylation of the ER gene may be a better

predictor of PR expression. In this pilot study we attempt to determine the rate of PR methylation in commonly used mammary derived cell lines (both normal and cancerous) as well as clinically derived tumor samples.

Methods: The frequency of PR-A and PR-B promoter methylation was tested by methylation specific PCR in *1)* five normal mammary epithelial cell strains and 11 cancerous mammary cell lines (table 1) and *2)* 16 primary breast tumors (table 2). Mammary epithelial cell strains and primary breast tumor DNA underwent bisulfite treatment prior to PCR-based analysis of methylation status. Primers were optimized to detect 5 methylated cells in 104 non-methylated controls cells.

***Results*:**

Table 1: Progesterone receptor methylation profiling of common and unique breast cell lines.

Cell Lines	ER	PRA-M	PRA-U	PRB-M	PRB-U
AG 32		+	+	+	+
AG 34		+	+		+
HMEC 15			+		+
HMEC 16			+		+
MCF 10A	-	+	+	+	+
E6L	-	+	+	+	+
SR	-	+	+	+	+
MCF7 β2	+		+		+
MCF7 SN			+		+
T47D	+		+		+
ZR751	+		+		+
MDA231	-	+	+		
MDA435	-	+	+	+	+
HS578T	-	+	+	+	+
BT474	+	+	+	+	+
DK	-		+		+

The PR-A promoter was methylated in 60% of the normal mammary cell strains and in 54% of breast cancer cell lines. The PR-B promoter was methylated in 40% of the normal mammary epithelial strains and in 45% of breast cancer cell lines. Most of the cancerous mammary cell lines that were ER negative were methylated for both PR-A and PR-B.

Table 2: Methylation profile of tumor samples.

Tumors	ER-M	ER-U	PRA-M	PRA-U	PRB-M	PRB-U
1	+	+	+	+		+
2			+			
3		+	+			
4			+	+		+
5		+	+	+		
6	+	+	+			+
7		+	+	+		+
8		+	n/a	n/a	n/a	n/a
9		+	+			
10	+		+	+	+	+
11		+	+			+
12	+					
13	+					
14		+				
15		+				
16	+	+	+			
17		+	+			

PR-A methylation was observed in 81% of the primary breast tumors. In contrast, there was only 12% incidence of PR-B promoter methylation and 37% incidence of methylation of the ER receptor promoter (*ESR1* promoter) in the same primary breast tumors.

Conclusion: Our initial results suggest that the PR-A promoter may be methylated at an earlier stage in mammary carcinogenesis than the PR-B or ER promoter. Future research will involve monitoring PR methylation status using RPFNA samples from a cohort of women at high-risk for developing breast cancer.

SPECIFIC AIM II: ***Are RPFNA cells isolated from patients that have persistent atypia following Tam chemoprevention resistant to estrogen-mediated activation of the ERE***

Evaluation of Breast-Tissue Estradiol and Triglycerides in a High-Risk Cohort:

We evaluated RPFNA and patient-matched serum estradiol and triglyceride levels and compared them to patient body-mass index (BMI), menopausal status and Masood cytology index. Estradiol and triglyceride levels were evaluated using an ELISA kit (ADI, San Antonio, TX) and colorimetric assay (TR-0100, Sigma-Aldrich), respectively. All assays were done in duplicate. Body weights and heights were clinically measured on a platform scale with a fixed stadiometer. Estradiol measurements were performed on 81 samples, Triglyceride values were determined for 73 samples and BMI was established for 74 samples. Masood cytology was determined by a single, dedicated, pathologist.

Methods: *Informed Consent:* This study was conducted among 47 women who sequentially underwent RPFNA under an IRB approved protocol at Duke University Medical Center from January 2006 to January 2007.

Eligibility: Women were required to have at least one of the following major risk factors for breast cancer: 1) 5-year Gail risk calculation $\geq$ 1.7% or 2) prior biopsy exhibiting atypical hyperplasia, lobular carcinoma *in situ*, or contralateral ductal carcinoma *in situ*. Samples from

BRCA1/2 mutation carriers were not collected to ensure the maximum uniformity of the cohort. Women were considered to be peri- menopausal, as defined by < 6 menstrual periods/year in the absence of pregnancy, polycystic ovarian syndrome, or thyroid disorder or post-menopausal as defined as no menses for >12 months in the absence of pregnancy and/or status post surgical removal of both ovaries, respectively. Socio-demographic variables (age, race), hormone-replacement (HRT)-use, and family history of breast cancer were collected. Given the potential for HRT to serve as a confounder of an investigation focused on body weight, steroid levels and breast cancer risk, all women currently using HRT on a routine basis were excluded from the analyses.

*Study Population***:** This study examined banked samples from 48 unique patients that provided 81 breast samples. For the purpose of this analysis, RPFNA samples obtained from different breast in the same individual were counted as separate samples. Samples were classified by a patient's menopausal status, BMI, and Masood Cytology Index. 36 patients were classified as pre-menopausal. This group contributed 63 breast RPFNA samples. The remaining 12 patients were classified as peri/post-menopausal and contributed 18 breast RPFNA samples. From these patients 33 underwent bilateral RPFNA and 15 underwent single breast RPFNA. Estradiol determination was conducted on 81 RPFNA samples, triglyceride (TG) determination was conducted on 73 samples and 74 samples were used for the correlation with BMI.

BMI Calculation: Body weights and heights were clinically measured on a platform scale with a fixed stadiometer; BMIs were calculated using the NIHLBI calculator (http://nhlbisupport.com/bmi/bmi-m.htm) and the standard cutpoint of 25 was used to dichotomize normal weight from overweight women.

RPFNA: RPFNA was performed as previously published. Slides for cytology were prepared in the laboratory of Dr. Carol Fabian. In subjects with prior invasive cancer or ductal carcinoma *in situ*, only the contralateral breast was aspirated. A minimum of one epithelial cell cluster with at least ten epithelial cells was required to sufficiently determine pathology; the most atypical cell cluster was examined and scored. Cells were classified qualitatively as non-proliferative, hyperplasia, or hyperplasia with atypia. Cytology preparations were also given a semiquantitative index score through evaluation by the Masood Cytology Index. As previously described, cells were given a score of 1-4 points for each of six morphological characteristics that include cell arrangement, pleomorphism, number of myoepithelial cells, anisonucleosis, nucleoli, and chromatin clumping; the sum of these points computed the Masood score: ≤10, non-proliferative (normal); 11-13, hyperplasia; 14-17 atypia; >17 suspicious cytology. The number of epithelial cells was quantified and classified as <10 cells (insufficient quantity for cytological analysis), 10-100 cells, 100-500 cells, 500-1,000 cells, 1,000-5,000 cells, and >5,000 cells. Morphological assessment, Masood Cytology Index scores, and cell count were assigned by a blinded, single dedicated pathologist.

RPFNA extracellular fluid and adipose collection: The RPFNA aspirate provides not only mammary epithelial cells but also associated immune cells, stromal cells, adipocytes, and extracellular fluid. As in prior studies, the RPFNA aspirate was placed in 10 ml modified Cytolyte^{tm} (9 ml cytolyt + 1 ml formalin) (Cytyc UK, West Sussex, UK). The solution is kept at room temperature overnight. The next day cells are mixed by pipetting up and down and 2/3 of the RPFNA sample transferred to a new 15 ml polypropylene centrifuge tube. The cells are washed (3-7 times) until the pellet is white. Cells are then spun in a Beckman CS-6R Centrifuge

at 25^0C at 2000 rpm for 10 min. Finally, most of the supernatant is removed with a 10 ml pipet and put into a new 15 ml conical (labeled with DU#) and samples were stored at -80 C for subsequent evaluation.

Estradiol Determination: Serum and solubilized RPNFA-EF/A estradiol concentrations in the were quantitated using an ELISA kit according to manufactures instructions (ADI, San Antonia, TX). Briefly, 50 µl of standards, control, and serum or RPFNA-EF/A samples were pipetted into anti-Mouse IgG coated wells in duplicate. 100 µl of Estradiol Enzyme Conjugate solution was added into each well and the plate was incubated at room temperature for 60 minutes on a plate shaker. The plate was then washed three times with wash buffer before the addition of 150 µl of HRP substrate (TMB) to each well. Plates were then incubated at room temperature for 10 min and the reaction was stopped with the addition of 50 µl of stopping solution. Absorbance was then measured at 450 nm (Perkin Elmer)

Triglyceride Determination: Briefly, 0.8 ml of the Free Glycerol Reagent was pipetted into each cuvette to which 10 µl (0.01 ml) of water, Glycerol Standard, and sample (serum or RPFNA-EF/A) was added. Reactions were then incubated at ambient temperature for 15 minutes. The initial absorbance (IA) of Blank, Standard, and Sample were then read at 540 nm versus water as the reference. Next 0.2 ml of the reconstituted Triglyceride Reagent was added to each cuvet, mix, and incubated 15 minutes at ambient temperature. The final absorbance (FA) of Blank, Standard, and Sample were read at 540 nm versus water as the reference.

Statistics: All statistical analysis was conducted using SAS v.9.0.

Results:

Associations of estradiol and triglyceride with menopausal status: Eighty-one RPFNA samples were tested from 48 unique patients. For the purpose of this analysis, RPFNA samples obtained from different breast in the same individual were counted as separate samples. There were no significant differences in mean RPFNA-EF/A E2 or RPFNA-EF/A TG concentrations between Pre- and Peri-menopausal women (525.76 mg/ml; n=63 and 542.22 mg/ml; n=18, respectively; p=0.166). However, there was a significant decrease in the mean ratio (E2/TG) for RPFNA-EF/A in Pre-menopausal women compared to perimenopasual women (649.88 mg/ml; n=56 and 576.36 mg/ml; n=17 respectively; p=0.026). There was no significant difference in the mean serum E2 or serum TG levels between premenopausal and perimenopausal women. (190.39 mg/ml; n=34 and 144.14 mg/ml; n=7 respectively; p=0.143). The ratio E2/TG was not significantly different in serum samples between premenopausal and perimenopasual samples (247.90 mg/ml and 123.65 mg/ml, respectively). Table 1.

*Association Between triglyceride and estradiol levels***:** The paired concentrations of RPFNA-EF triglyceride and estradiol had a weak but statistically significant negative correlation (Pearson's correlation coefficient, r=-0.26, p=0.028). Therefore, as triglyceride levels increase estradiol concentrations tend to decrease, vice versa (Table 1).

Associations with Body Mass Index (BMI): Forty-one RPFNA samples were stratified with respect to BMI. Seventy-four samples from 41 patients were evaluated. For the purpose of this analysis, RPFNA samples obtained from different breasts in the same individual were counted as separate samples. Women with a BMI below 20.6 had significantly lower mean RPFNA-EF E2 levels compared with those with a BMI greater than 20.6 (377.55 mg/ml; n=21 and 458.31

mg/ml; n=53 respectively; p = 0.004). There were no significant differences between RPFNA-EF E2 or Serum E2 for those classified as obese (BMI >25;n=28) versus those classified as not obese (BMI <25; n=46), p=0.725). There was no significant difference between RPFNA-EF TG or Serum TG for those classified as obese versus those classified as not obese (p=0.569). Table 1.

Table 1. Comparison of serum levels of estradiol (E2), triglyceride (TG), and ratios of average E2: triglyceride levels in pre- and post-menopausal cohorts and relative BMI cohorts.

	RPFNA-EF Values			Serum Values		
	E2 (mg/ml)	TG (mg/ml)	E2/TG (mg/ml)	E2 (mg/ml)	TG (mg/ml)	E2/TG (mg/ml)
Premenopause	525.76	0.809	649.88	190.39	0.768	247.90
Perimenopause	542.22	1.012	541.57	144.14	1.147	125.65
BMI<20.6	377.55	0.879	429.52	181.67	0.540	336.28
BMI>20.6	458.31	0.926	494.93	185.87	1.024	181.51

Associations with Masood index: The association between RPFNA-EF E2 and RPFNA-EF E2/TG and Masood Cytology Index was tested. The distribution of Masood Cytology Scores is depicted in Table2 and Figure 3. Seventy samples were tested from 43 women. RPNFA samples obtained from different breasts in the same individual were analyzed as separate samples. There was a significant positive correlation between Masood index and RPFNA-EF E2 and Masood index and RPFNA-EF E2/TG ratio (r = 0.256, p = 0.034 and r = 0.270, p = 0.031, respectively). There is a significant increase in mean RPFNA-EF E2, Serum E2, and RPNFA-EF E2/TG ratio levels from those with Masood index scores less than 13 versus those with a score greater than or equal to 13 (p-values = 0.004, 0.039 and 0.026, respectively). Table 2 and Figure 1.

Table 2. Comparison of E2 levels in RPFNA versus serum samples and the relationship between triglyceride levels for these same patients based on Masood score

		RPFNA Values			Serum Values		
Masood	**n**	**E2**	**TG**	**E2/TG**	**E2**	**TG**	**E2/TG**
10	6	314.74	1.324	237.77	160.2	0.566	282.99
11	3	354.79	1.034	342.96	188.76	1.191	158.55
12	7	340.87	0.979	348.05	158.33	0.789	200.67
13	16	464.86	0.837	555.22	185.48	0.758	244.76
14	12	478.43	0.926	516.82	188.48	0.69	273.27
15+	16	465.81	0.988	471.61	215.18	1.253	171.68

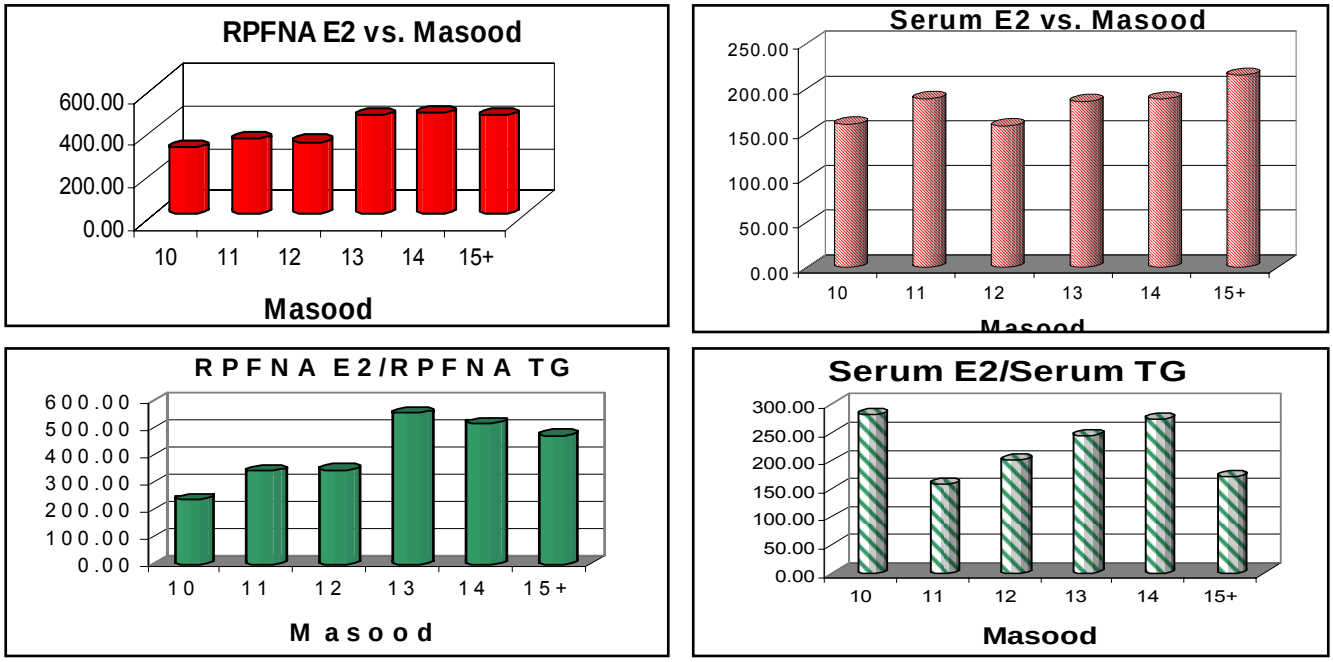


Figure 1. Correlation between E2:TG levels in RPFNA-EF/A versus serum using cytology. In the RPFNA-EF/A there is a noticeable increase in E2:TG levels between masood scores 12 and 13. The trend shows a plateau from 10-12 and a plateau in E2:TG level at 13 and above.

Conclusion:

In general we found that breast tissue levels of estradiol were not significantly different based on menopausal status. The use of triglyceride values for correcting estradiol levels had a significant impact on the general results of this study. The effects of BMI were only at the bottom quartile, in general prior correlations between BMI and breast cancer risk have been associated with BMI's greater than 25. As demonstrated in previous studies looking at nipple aspirate fluid the breast level steroid concentrations are significantly higher than those observed in the serum. Our demonstration that these levels did not change with menopausal status may indicate an important prolonged role for estradiol in the general maintenance of the breast tissue. Likewise though this may suggest that overall the role for estradiol in cancer initiation/progression may be overstated. However, interpretation is made difficult by the fact that our cohort consists of women who have already been designated as high risk. Because of this we also evaluated the effect of masood score that is why significant increase at 13 is important, masood 13 is generally considered the cutoff point for the start of cytological atypia.

Steroid detection and profiling in samples using gas-chromatography-mass-spectrometry (GC-MS):

To enhance our ability to distinguish the effects of estradiol and tamoxifen on cytological changes we have been working to establish a protocol for the detection and quantation of tissue-level steroids using gas-chromatography/mass-spectrometry (GC-MS).

Methods:

Instrumentation: A Shimadzu GC-2010 gas chromatograph was used for analyzing our prepared samples. Samples were loaded into an autosampler and Shimadzu "LabSolutions" GC-MS Solutions data program was used for data analysis. Column oven temperature was set at 80.0 ºC and injection temperature was held at 280.0 ºC. Ion source temperature was set to 250 ºC and interface temperature was held at 280.0 ºC. Splitless injection was employed. Run time did not exceed 15 minutes with expected retention time of steroid and metabolites approximated around normal retention time of estradiol (12.3 seconds).

Materials: LC-MS grade ethyl acetate and HPLC/GC grade isopropanol were purchased from Burdick & Jackson (Morristown, NJ). Absolute diethyl ether was purchased from EMD. Absolute EtOH was purchased from Aldrich. Silylation grade derivatizing solvents ACN and pyridine were purchased from Pierce (Rockford, IL). Derivatizing agent MSTFA was purchased from Agilent (Santa Clara, CA) and was stored in airtight vials under the hood. Estradiol was purchased from Aldrich at 98% β-estradiol and was kept in a desiccator and refrigerated for storage. GC-MS sample viles were purchased from Agilent. All glassware, syringes, and plastic storage bottles were washed thoroughly with ethyl acetate (x2) and isopropanol (x1) before use to prevent interferences and/or contamination from plasticizers or other pollutants in spectra. Glass tubes were used for storing standard solutions. Glass syringes were used for solvent transfer.

Standard sample preparation: Each standard sample was prepared by serial dilution using EtOH. Standard concentrations were prepared at 3.20 ng/ml, 800 pg/ml, 300 pg/ml, 100 pg/ml, and 20 pg/ml. Solutions were vortexed for 30 seconds between each dilution to ensure solvation. GC-MS spectra were obtained to confirm concentrations and detection of each standard solution before derivatization.

Derivatization: All derivatization reactions were performed underneath a hood. For standard solutions, 200 µl aliquots of each prepared standard were transferred to a 2.0 ml GC-MS glass vile. All samples were then placed under N_2 or Ar and re-suspended in 200 µl ACN:Pyridine (9:1) along with 10µl of derivatizing agent (MSTFA). Samples were capped tightly and placed on a heating block at 60-65 ºC for 60 minutes. After cooling to room temperature excess solvent and derivatizing agent were evaporated under N_2 or Ar. The resultant residue was dissolved in dehydrated diethyl ether and transferred to reacti-vials inside the GC-MS vials and inserted into autosampler for GC-MS analysis.

Results:

For analytical purposes, peak area was used rather than peak height in order to obtain a standard curve function suitable for clinical use. Standard solution spectra correspond to values set by ELISA (see previous section). The spectra (particularly for underivatized estradiol standard solutions) show increasing fragment peak area with concentration. The spectra for underivatized (figures 2.1a, 2.2a, 2.3a, 2.4a and 2.5a) estradiol samples indicate that some contamination with either a plasticizer or silicon leakage from the GC-septum occurred. Since the fragment peaks are not well-defined, integration was approximated using retention time range rather than exact peak width. Derivatized samples show much more purity (figures 2.1b, 2.2b, 2.3b, 2.4b, and 2.5b). The lack of underivatized estradiol as well as the lack of half-derivatized estradiol in the derivatized spectra indicates that the derivatizing reaction went to completion in these solutions. Fragment peak areas were still obtained as interferences did not hinder fragmentation but rather the quality and sharpness of the fragment peaks. Using the integrated areas for the derivatized

and underevatized peaks we generated standard curves for future use in evaluating our RPFNA samples on the GC-MS and for comparison with results from ELISA based determinations (Figure 3).

a)

b)

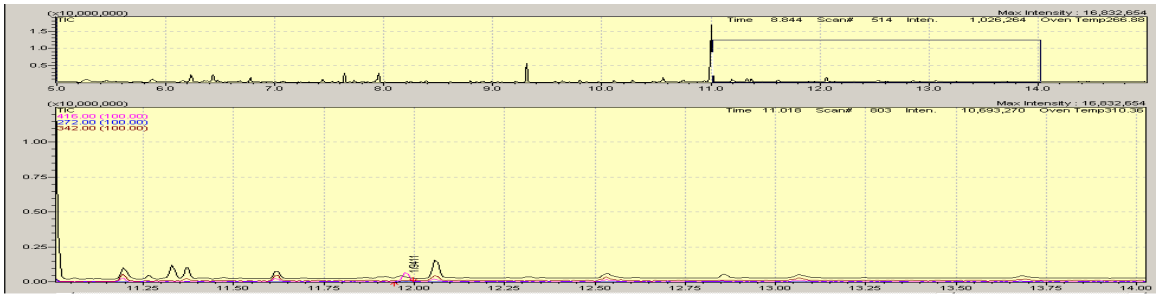
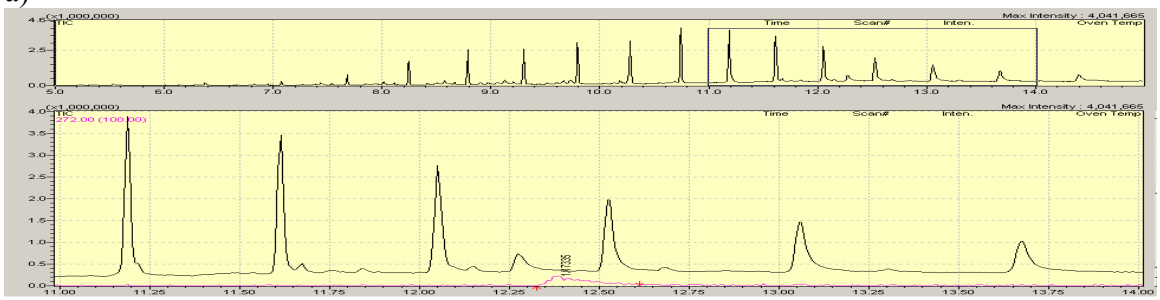


Figure 2.1 GC-MS spectra for standard [Estradiol] =20 pg/ml a) underivatized and b) derivatized.

a)



b)

Figure 2.2 GC-MS spectra for standard [Estradiol] =100 pg/ml a) underivatized and b) derivatized.

a)

b)

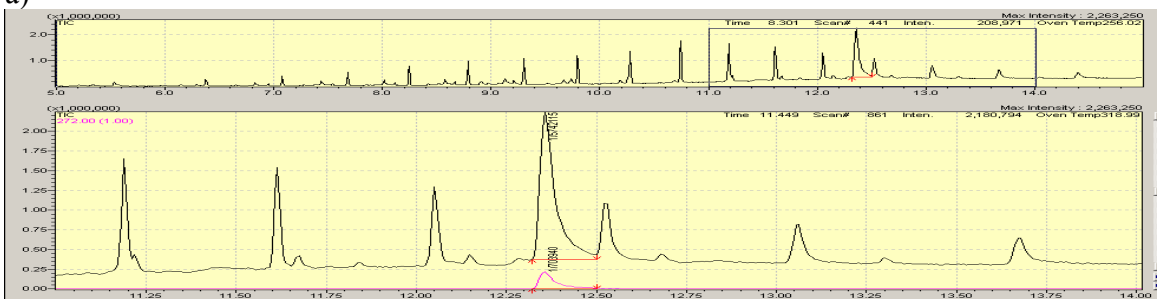
Figure 2.3 GC-MS spectra for standard [Estradiol] =300 pg/ml a) underivatized and b) derivatized.

a)

b)

Figure 2.4 GC-MS spectra for standard [Estradiol] =800 pg/ml a) underivatized and b) derivatized.

a)



b)

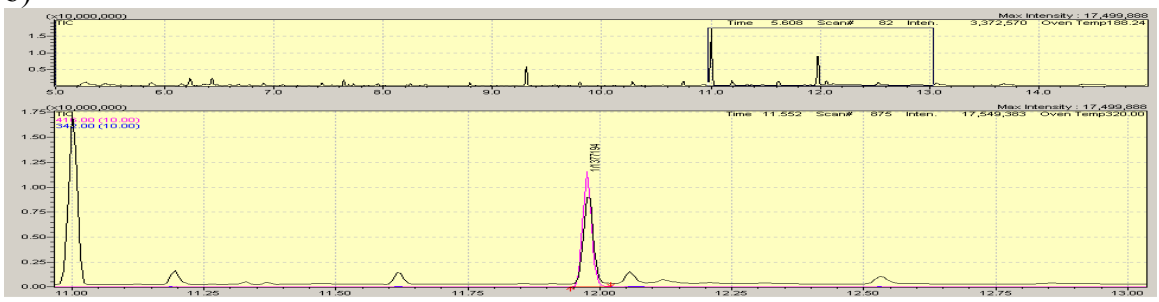
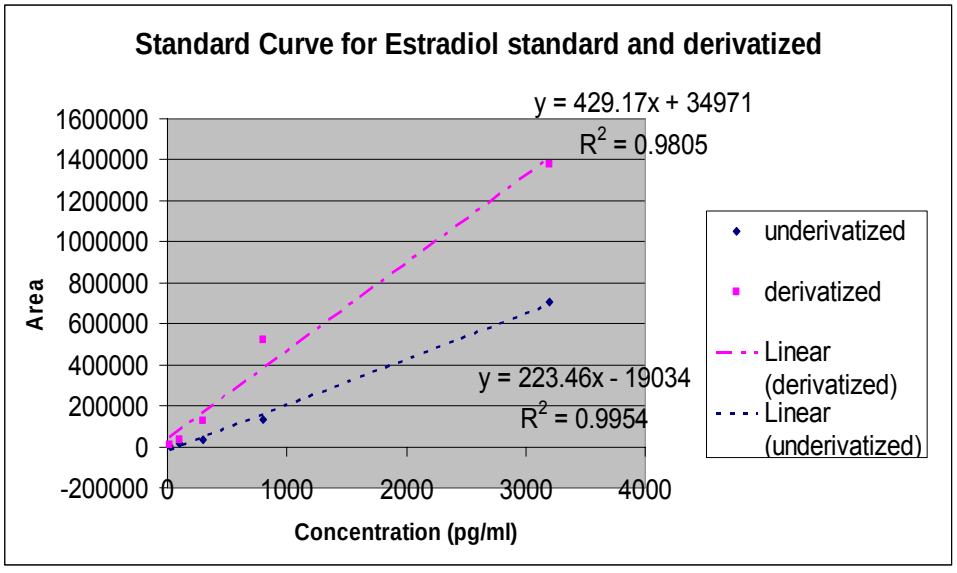


Figure 2.5 GC-MS spectra for standard [Estradiol] =3200 pg/ml a) underivatized and b) derivatized.

a)



b)

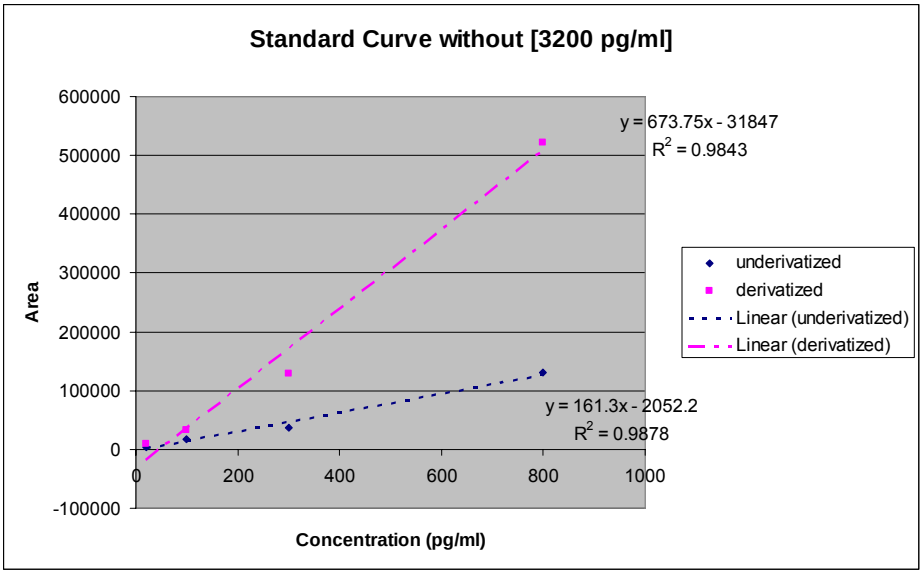


Figure 3. Standard curve for derivatized [estradiol] for a) all data points and b) at the lowest limit of sensitivity. The standard curve found is (b) is more useful clinically speaking since 3200 pg/ml is outside the range of values to be encountered in early detection. The correlation coefficient improves somewhat for this plot as well.

Conclusion:

GC-MS spectra show a great deal of background noise for underivatized samples, making it difficult to analyze our lowest concentration (20 pg/ml). With further testing of solvents and extraction protocols, as well as improved derivatization technique, analysis using GC-MS shows promise for further development in steroid profiling. The source of the noise for the underivatized estradiol standards was undeterminable. Other spectra were cross-checked for the same time period which did not show the same background noise, eliminating column leakage as the source. All solvents were GC-MS grade and were checked in GC-MS upon receipt, though contamination could have occurred between that time and usage. Producing spectra proved to be a more arduous task than predicted. The protocol for creating standard solutions was straightforward, but complications arose due to the highly lipophilic nature of estradiol (as well as estrone).

Key Research Accomplishments

- Human primary stromal cell culture – Established a primary cell line for evaluation of changes to stromal cells.
- Steroid determination from RPFNA – As part of a senior thesis project we established a SOP for the evaluation of steroids in the microenvironment of the breast using gas-chromatography/mass spectronomy.
- Approval for use of human subject material – In March of 2007 we received final approval from the Human Research Protection Office in the Office of Research Protections to use previously acquired samples for our evaluations.
- Steroid measurement – RPFNA samples have been evaluated for the concentration of estradiol and triglyceride using ELISA's. Testing and evaluation of these samples will be

completed in the summer of 2007 with intended manuscript submission of early fall 2007. Results of this dataset will also be presented at two upcoming meetings – AACR – BCERC

- PR methylation status – designed and validated PCR primers for the detection of methylation of PRA and PRB promoters in our RPFNA samples. Results of this investigation will be included with other data for submission of publication in early fall 2007.

Reportable Outcomes

- Presentation of PR data at annual Duke Comprehensive Cancer Center Meeting
- Results of GC/MS project were part of a distinguished Senior Thesis

Conclusion

In this report we have demonstrated our ability to screen for methylation of progesterone receptors in cell lines as well as tumor samples. We will next incorporate the study of PR methylation into our examination of RPFNA samples and evaluate these results with the proposed examination of estrogen receptor methylation in the same samples. To enhance our understanding of the role of hormones and tamoxifen in altering binding partners of ERE's we have developed two protocols for establishing tissue level hormone concentrations. The first method uses standard ELISA kits and our initial results from studies of RPFNA samples demonstrate that there are steroid-cytology correlations which should be taken into consideration in study design. Additionally, we have made significant progress in developing a novel GC-MS method for simultaneous evaluation of multiple steroid types from our RPFNA samples. The ability to model a more complex steroid microenvironment will nhance our understanding of the how changes in the steroid environment effect recruitment of proteins to the response element.